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Detection of *Toxoplasma gondii* Oocysts in Drinking Water

JUDITH ISAAC-RENTON,[1]* WILLIAM R. BOWIE,[2] ARLENE KING,[3] G. STEWART IRWIN,[4] CORINNE S. ONG,[1] C. P. FUNG,[5] M. OMAR SHOKEIR,[1] AND J. P. DUBEY[6]

Department of Pathology and Laboratory Medicine[1] and Division of Infectious Diseases, Department of Medicine,[2] University of British Columbia, and Epidemiology Services[3] and Provincial Laboratory,[5] British Columbia Centre for Disease Control, British Columbia Ministry of Health, Vancouver, and Water Department, Capital Regional District,[4] Victoria, British Columbia, Canada, and Parasite Biology and Epidemiology Laboratory, Livestock and Poultry Sciences Institute, Agricultural Research Service, U.S. Department of Agriculture, Beltsville, Maryland[6]

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The world's largest outbreak of waterborne toxoplasmosis occurred in a municipality in the western Canadian province of British Columbia. When drinking water emerged as a possible source of infection during the outbreak investigation, a laboratory method was needed to attempt detection of the parasite, *Toxoplasma gondii*. The method developed was based on the current U.S. Environmental Protection Agency method for detection of *Cryptosporidium* oocysts. Collection of large-volume drinking water samples and cartridge filter processing were unchanged, although identification of *Toxoplasma* oocysts in the filter retentate was carried out by using a previously described rodent model. Validation of the method developed was tested by using oocysts from a well-characterized *Toxoplasma* strain.

A community outbreak of toxoplasmosis in western Canada prompted extensive epidemiological investigations. One hundred acute cases and 12 congenital cases of infection with *Toxoplasma gondii* were identified during these investigations. The diagnostic laboratory methods and epidemiological investigations are described in detail elsewhere (3). Briefly, a case-control study of symptomatic cases, a case-control study of women enrolled in the toxoplasmosis screening program, and geographical mapping of acute cases were carried out. A clustering of cases was noted in the central area of greater Victoria, British Columbia. Eighty-eight percent (83 of 94) of the persons residing in the greater Victoria area with acute infections lived in an area of the city receiving its water from one of the two treatment plants (disinfection only). When drinking water emerged as a possible source of infection, a laboratory method was needed to attempt to detect *T. gondii* oocysts in water samples. Based on the method used to detect *Cryptosporidium* oocysts (1), the new procedure used to detect the parasite in the concentrate differed as described below. We propose that this method could be useful in the investigation of waterborne transmission of toxoplasmosis parasites, which were not widely recognized as being spread by this route prior to the Canadian municipal outbreak (3). The purpose of the present communication is to describe this method.

The Humpback Reservoir, identified during the investigation as the most likely source of *T. gondii* oocysts, supplied one of the two municipal drinking water treatment plants. A sampling site was set up at the point where water left this reservoir to enter the Humpback treatment plant. One or two samples per week (a total of six water samples) were collected from the Humpback Reservoir over 4 weeks. Water samples were also collected on the same days as these samples but from the reservoir supplying the other treatment plant but not implicated in the outbreak. All samples were raw water collected before the water entered the chloramination treatment plants.

A method was developed that is similar to the method used for collection and processing of water samples for *Cryptosporidium* sp., a protozoan related to *T. gondii*. The method is as follows. Large volumes (the target volume was 1,000 liters; all volumes were greater than 700 liters) were collected for each sample. Since *T. gondii* oocysts (10 to 12 μm in diameter) are larger than *Cryptosporidium* oocysts (3 to 5 μm in diameter), samples were collected by using the recommended (1) 1-μm nominal porosity, orlon-wound filter cartridge. A large-volume sample of water was passed through the filter housed in a portable collection device by keeping the flow rate at 4 to 10 liters/min. Filters were separated from the collection apparatus and transported in coolers (on ice).

On arrival at the laboratory, filters were cut into four parts and parasites were eluted by washing in phosphate-buffered saline–0.01% Tween 80–0.01% sodium dodecyl sulfate. After centrifugation (1,050 × *g*, 4 min, 4°C), sediments from individual test tubes were pooled and the pellet was resuspended in distilled water to a volume of 20 ml. This suspension was layered onto a series of conical test tubes containing 30 ml of a Percoll-sucrose solution (specific gravity, 1.15), and the test tubes were centrifuged (1,050 × *g*, 10 min, 4°C). The top 25 ml was then aspirated off into another test tube and diluted fourfold with phosphate-buffered saline–0.01% Tween 80–0.01% sodium dodecyl sulfate. Following a final centrifugation step (1,050 × *g*, 10 min, 4°C), the supernatant was aspirated off and the sediment was kept for sporulation and inoculation.

An aeration procedure was required to sporulate, and therefore render infectious, any *T. gondii* oocysts in this sediment. This procedure was carried out as follows. One milliliter of sediment was mixed with 9 ml of 2% sulfuric acid and slowly agitated on a rocking platform (Red Rocker; Hoefer Scientific, San Francisco, Calif.) at room temperature for 7 days. The suspension, neutralized by using 3.3% sodium hydroxide (2% phenol red pH indicator), was centrifuged (1,050 × *g*, 10 min, 4°C), the supernatant was aspirated down to 1 ml per tube, and the pellets were resuspended in sterile saline. Suspensions were kept at 4°C until inoculation into mice was carried out.

Female Swiss-Webster mice weighing 25 to 30 g each were

* Corresponding author. Mailing address: Division of Microbiology and Infection Control, Department of Pathology and Laboratory Medicine, Laurel Street Pavilion, Vancouver General Hospital, 855 West 12th, Vancouver, British Columbia, Canada V5Z 1M9. Phone: (604) 875-4631. Fax: (604) 875-4359. E-mail: isaacren@unixg.ubc.ca.

ear tagged and housed separately in sterilized cages with HEPA filters. They were given irradiated food and sterile water ad libitum. For each water sample, four test mice were inoculated. For each batch of samples inoculated, three negative control mice were also inoculated. One-half milliliter of the aerated, well-mixed, resuspended sample sediment was inoculated by gavage (with a sterile gavage needle and plastic tubing) into each of the four test mice (a total of 2 ml was inoculated per sample). The bedding was discarded and the cages were sterilized at 24 h postinoculation. Each of the three negative control mice was inoculated with 0.5 ml of sterile saline on the day of test sample inoculation.

Mice were observed twice daily for 60 days or until death. If a mouse died, an autopsy was carried out. Touch preparations and formalin fixing of appropriate tissues (mesenteric lymph nodes and lung and cardiac tissues) were performed. Touch preparations fixed with methanol were stained with Giemsa stain for examination by microscopy. Tissues fixed in 10% buffered formalin were embedded in paraffin by using standard histological procedures. After embedding, specimens (sectioned at 5-μm intervals) were stained with Giemsa. Additional sections were also stained with hematoxylin and eosin to observe the lesions. Slides were examined microscopically, including a search for parasites by oil immersion (magnification, ×1,000). *Toxoplasma* tachyzoites were identified when crescentic, oval, or fusiform nucleated organisms measuring approximately 2 by 6 μm were seen. If a mouse survived for 60 days, blood was collected for serological testing (modified agglutination test [MAT]) for toxoplasmosis (4, 5) in the U.S. Department of Agriculture Laboratory at Beltsville, Md. Sera were not collected from rodents that died.

To evaluate this method, oocysts of *T. gondii* VEG (human source strain) were used to inoculate Swiss-Webster mice (5). A series of dilutions ranging from 2.5×10^4 to less than 1 sporulated oocyst were made by using sterile saline. For each dilution, 0.5 ml of a well-mixed suspension was given by gavage to each of five test mice. Four negative control mice were also inoculated with 0.5 ml of sterile saline on the day test mice were inoculated. Mice were caged separately and observed twice daily. If a mouse died, tissue specimens were processed as noted above. Sera were obtained from all surviving mice, diluted 1:25, and tested for *T. gondii* by MAT.

Eleven raw water samples were tested, six from the implicated water source (Humpback Reservoir) and five from the nonimplicated reservoir. The mean volumes collected were 1,051 liters for Humpback Reservoir samples and 968 liters for samples from the second reservoir. A total of 56 mice were inoculated with samples, including 24 mice given Humpback reservoir samples, 20 mice given second-reservoir samples, and 12 mice used as saline-treated negative controls. At the end of 60 days, none of these mice had died. All were serologically negative for toxoplasmosis.

A total of 34 mice were inoculated in the experiments using *T. gondii* VEG. Results are summarized in Table 1. Twenty-three mice showed evidence of infection. Sixty-five percent (15 of 23) of the infected mice died between 6 and 11 days postinoculation. Toxoplasmosis was documented in the animals that died by verifying the presence of the parasite; parasites were observed in 100% (15 of 15) of lung specimens, 86% (12 of 14) of cardiac specimens, and 73% (11 of 15) of mesenteric lymph nodes. Necrotizing lymphadenitis was observed in the 11 nodes in which *T. gondii* tachyzoites were identified, while significant histopathology was absent in the 15 lung and 12 cardiac tissue specimens in which parasite tachyzoites were seen. Infection was documented in 8 of the 19 surviving mice by positive serology (MAT) results. As seen in Table 1, none of the saline-treated control mice showed evidence of infection and none of the mice inoculated with the lowest dilution (less than 1 oocyst) had evidence of toxoplasmosis. Inoculation of an estimated 2.5 oocysts resulted in infection in three of five mice; five of five mice receiving 25 oocysts became infected. This experiment is consistent with previous descriptions of this rodent model of toxoplasmosis (5, 6), in which the sensitivity and specificity are well described.

TABLE 1. Response of rodents to inoculation with sporulated *Toxoplasma* strain VEG oocysts

No. of oocysts inoculated	No. of mice inoculated	No. of mice/total showing:		
		Death (mean no. of days postinoculation)	Positive tissues (tachyzoites present)	Positive serology result
25,000	5	5/5 (7)	5/5	ND[a]
2,500	5	5/5 (8)	5/5	ND
250	5	5/5 (10)	5/5	ND
25	5	0/5	ND	5/5
2.5	5	0/5	ND	3/5
0.25	5	0/5	ND	0/5
0 (negative controls)	4	0/4	0/2	0/4

[a] ND, test not done.

As noted in the description of this outbreak (3), investigations implicated the Humpback Reservoir as the source of the *T. gondii* oocysts. The epidemic curve (7) showed clusters of persons acutely infected during two periods of time preceded by peaks in rainfall and turbidity in this unfiltered drinking water supply. It was hypothesized that parasite contamination of the drinking water occurred during these periods of high runoff. It was further hypothesized that because the implicated reservoir was relatively small with a high turnover, sporulated oocysts from an infected feline(s), the definitive host for *T. gondii*, were carried into the municipal water distribution system. In retrospect, since collection of drinking water samples for laboratory testing was started approximately 12 weeks after the last human was infected, it was not surprising that attempts to confirm epidemiological findings by laboratory testing of drinking water samples were not successful. This large-volume water sampling method linked to a rodent model does, however, appear to have the potential to be a sensitive test for detection of *Toxoplasma* oocysts.

Clusters or outbreaks of toxoplasmosis have been infrequently described. Foodborne spread and waterborne spread have been reported (2, 8), although not previously in a municipal water supply. Where *Toxoplasma* contamination of a drinking water supply is suspected, the method described above may be useful in detecting oocysts of the parasite if samples are collected early in the outbreak investigation. It is therefore important that water purveyors and public health workers be aware that *T. gondii* is yet another protozoan parasite which may be transmitted through drinking water.

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REFERENCES

1. **American Society for Testing and Materials.** 1992. D-19 proposal P229. Proposed test method for *Giardia* cysts and *Cryptosporidium* oocysts in low turbidity water by a fluorescent antibody method, p. 925–935. *In* Annual book of ASTM standards, section 1, water and environmental technology, vol. 11.02. American Society for Testing and Materials, Washington, D.C.

2. **Beneson, M. W., E. T. Takafuji, S. M. Lemon, R. L. Greenup, and A. J. Sultzer.** 1982. Oocyst-transmitted toxoplasmosis associated with ingestion of contaminated water. N. Engl. J. Med. **300:**694–699.
3. **Bowie, W. R., A. S. King, D. H. Werker, J. Isaac-Renton, A. A. Bell, S. B. Eng, and S. Marion.** 1997. Outbreak of toxoplasmosis associated with municipal drinking water. Lancet **350:**173–177.
4. **Dubey, J. P., and G. Desmonts.** 1987. Serological responses of equids fed *Toxoplasma gondii* oocysts. Equine Vet. J. **19:**337–339.
5. **Dubey, J. P., C. A. Speer, S. K. Shen, O. C. H. Kwok, and J. A. Blixt.** 1997. Oocyst-induced murine toxoplasmosis: life cycle, pathogenicity, and stage conversion in mice fed *Toxoplasma gondii* oocysts. J. Parasitol. **83:**870–882.
6. **Dubey, J. P., J. K. Lunney, S. K. Shen, O. C. H. Kwok, D. A. Ashford, and P. Thulliez.** 1996. Infectivity of low numbers of *Toxoplasma gondii* oocysts to pigs. J. Parasitol. **82:**438–443.
7. **Irwin, G. S., A. S. King, D. H. Werker, A. A. Bell, R. Gill, J. Isaac-Renton, S. B. Eng, T. Johnstone, W. R. Bowie, S. A. Marion, D. Roscoe, A. Burnett, F. Jagdis, P. MacLeod, and J. Hull.** An outbreak of toxoplasmosis associated with municipal drinking water—water quality and water supply aspects. *In* Proceedings of the 7th National Drinking Water Conference, in press.
8. **Won-Young, C., H. Nam, N. Kwak, W. Huh, Y. Kim, M. Kang, S. Cho, and J. P. Dubey.** 1997. Foodborne outbreaks of human toxoplasmosis. J. Infect. Dis. **175:**1280–1282.